

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problems Mailbox.**

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12P 21/00, C12N 1/20, 15/00, G01N 33/53, C07H 21/04, C07K 1/00, 14/52, A61K 45/05, 38/19, 38/00	A1	(11) International Publication Number: WO 97/42340 (43) International Publication Date: 13 November 1997 (13.11.97)
(21) International Application Number: PCT/US97/07521 (22) International Filing Date: 2 May 1997 (02.05.97) (30) Priority Data: 60/016,899                      6 May 1996 (06.05.96)                      US 9610995.4                      24 May 1996 (24.05.96)                      GB (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CASKEY, C., Thomas [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HESS, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). HEY, Patricia [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). PHILLIPS, Michael, S. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).	(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.	
(54) Title: OB RECEPTOR ISOFORMS AND NUCLEIC ACIDS ENCODING THEM (57) Abstract <p>The ob receptor has numerous isoforms resulting from alternative splicing; three novel isoforms, designated c', f, and g are disclosed. The nucleic acids encoding these isoforms are taught. Also part of the invention are vectors containing the nucleic acid encoding the receptors, host cells transformed with these genes, and assays which use the genes or protein isoforms.</p>		

# FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

- 2 -

Furthermore, the recent cloning of the human receptor for the leptin, the ob-receptor (OB-R), reveals that it is transcribed in the hypothalamus (Tartaglia *et al.* 1995, *Cell* 83:1263-1271; Stephens *et al.* 1995, *Nature* 377: 530-532). In addition, a mutation that  
5 results in premature termination of the long-form of the mouse OB-R, which is preferentially expressed in the hypothalamus, appears to be responsible for the obese phenotype of the *db/db* mouse (Lee *et al.* 1996, *Nature* 379:632-635; Chua *et al.* 1996, *Science* 271:994-996; and Chen *et al.* 1996, *Cell* 84:491-495).

10 The OB-R from wild type (lean) rats and from rats having the *fatty* mutation (both heterozygous and homozygous *fa*) have been isolated and sequenced. (Patent Application Serial Nos. \_\_\_\_\_, Attorney Docket Nos. 19642PV and 19642PV2, filed February 22, 1996 and March 22, 1996, which are hereby  
15 incorporated by reference.)

Various isoforms of the OB-Rs have also been identified. These isoforms are due to alternative splicing. For example, in the mouse the a form has 5 amino acids following the Lysine at 889; the b form has 273 amino acids after Lysine 889; the c  
20 form has 3 amino acids after Lysine 889; and the d form contains 11 amino acids after Lysine 889.

It would be desirable to be able to further experiment with various isoforms in order to better understand obesity, and to be able to clone and produce novel *ob* receptor isoforms to use in  
25 assays for the identification of ligands which may be useful in understanding obesity and for its prevention and treatment.

#### DETAILED DESCRIPTION OF THE INVENTION

This invention relates to novel *ob* receptor isoforms  
30 designated c', f and g which are substantially free from associated membrane proteins. It also relates to substantially purified *ob* receptor isoform c', f and g proteins. These isoforms are present in various species, including rat, mouse and human.

- 3 -

Another aspect of this invention is to nucleic acids which encode OB receptor isoforms c', f or g. The nucleic acid may be any nucleic acid which can encode a protein, such as genomic DNA, cDNA, or any of the various forms of RNA. Preferably, the nucleic acid is cDNA.

This invention also includes vectors containing a OB-R isoform c', f or g gene, host cells containing the vectors, and methods of making substantially pure OB-R isoform c', f or g protein comprising the steps of introducing a vector comprising a OB-R isoform c', f or g gene into a host cell, and cultivating the host cell under appropriate conditions such that OB-R isoform c', f or g is produced. The OB-R isoform c', f or g so produced may be harvested from the host cells in conventional ways.

Yet another aspect of this invention are assays which employ OB-R isoform c', f or g. In these assays, various molecules, suspected of being OB-R isoform c', f or g ligands are contacted with a OB-R isoform c', f or g, and their binding is detected. In this way agonists, antagonists, and ligand mimetics may be identified. A further aspect of this invention are the ligands so identified.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is the amino acid sequence of wild type rat OB-R.

FIGURE 2 is the cDNA sequence of wild type rat OB-R.

FIGURE 3 is the cDNA sequence encoding rat isoform.

FIGURE 4 is the cDNA specific for Rat isoform c'.

As used throughout the specification and claims, the following definitions apply:

"Substantially free from associated membrane proteins" means that the receptor protein is not in physical contact with any membrane proteins.

"Substantially purified OB-receptor isoform c', f or g" means that the protein isoform is at least 90% and preferably at least 95% pure.

- 4 -

"Wild type" means that the gene or protein is substantially the same as that found in an animal which is not considered to have a mutation for that gene or protein.

5 "fa" means that the gene or protein is substantially the same as that found in a rat homologous for the *fatty* mutation.

"Substantially the same" when referring to a nucleic acid or amino acid sequence means either it is the same as the reference sequence, or if not exactly the same, contains changes which do not affect its biological activity or function.

10

It has been surprisingly found, in accordance with this invention that the OB-R exists in a large variety of isoforms, including three novel ones, form c', f and g. These isoforms apply to all species, but for convenience, throughout the specification and  
15 claims, numberings of amino acids and nucleotides will use the rat wild type sequences (FIGURES 1 and 2) as a reference. However, it is to be understood that this invention is not limited to rat wild type proteins and nucleic acids and specifically includes rat (wild type and *fatty*), mouse, and human OB-R isoform c', f and g proteins and  
20 nucleic acids.

OB-R isoform f differs from wild type protein in that after the Lysine at position 889 (referring to the rat sequence in FIGURE 1), there are six amino acids, ending at an Asparagine  
25 residue at position 895. In the cDNA, the codons are then followed by a Stop codon. One cDNA for rat isoform f is shown in FIGURE 3; this invention specifically includes all various cDNAs encoding an isoform f protein. The superscripted numbers refer to protein  
position numbers.

30

Lys<sup>889</sup> Iso<sup>890</sup> Met<sup>891</sup> Pro<sup>892</sup> Gly<sup>893</sup> Arg<sup>894</sup> Asn<sup>895</sup>

In the human isoform f, Lysine 891 corresponds to the rat Lysine 889, the same six amino acids follow Lysine 889.

In a particularly preferred embodiment of this invention, the OB-R isoform f is from rat origin.

- 5 -

OB-R isoform g differs from the wild type in that it is much shorter than the wild type sequence. The following eighteen amino acids are found at the beginning of the protein with the superscript numbers indicating their position. The Arginine at position 18 is spliced to a large fragment of the wild type molecule, beginning at the Proline at position 166 (in both mouse and human). This isoform then extends for the remainder of the wild type molecule.

Met<sup>1</sup> Phe<sup>2</sup> Gln<sup>3</sup> Thr<sup>4</sup> Pro<sup>5</sup> Arg<sup>6</sup> Ile<sup>7</sup> Val<sup>8</sup> Pro<sup>9</sup> Gly<sup>10</sup>  
His<sup>11</sup> Lys<sup>12</sup> Asp<sup>13</sup> Leu<sup>14</sup> Ile<sup>15</sup> Ser<sup>16</sup> Lys<sup>17</sup> Arg<sup>18</sup> Pro<sup>166</sup>...

After Pro<sup>166</sup>, the remainder of the protein may be the same as wild type, or, alternatively it could also contain another isoform variation, such as isoform a, b, c, d, e, or f.

A particularly preferred embodiment is the rat isoform g.

OB-R isoform c' is similar to the OB-R isoform c which was previously described [Lee *et al.*, *Nature* 379: 632-635]. After Lysine at position 889, it only has three amino acids, Val<sup>890</sup> Thr<sup>891</sup> Phe<sup>892</sup> Stop. As can be seen, isoform c' differs from isoform c in that the final amino acid is phenylalanine rather than valine found in isoform c. Further, there are untranslated sequences in the DNA encoding isoform c' which do not appear to be present in isoform c. The cDNA encoding the rat isoform c' is given in FIGURE 4. In humans, the Val, Thr, Phe follow Lysine 891.

One aspect of this invention is the molecular cloning of these various isoforms of OB-R. The wild type and *fa* receptor proteins contain an extracellular, a transmembrane domain. In the rat, the extracellular domain extends from amino acids 1-830; the transmembrane domain is from amino acids 839-860; and the cytoplasmic domain is from amino acids 860-1162. Similar domains have been identified for the mouse and human proteins. This

- 6 -

invention also includes isoform c', f and g proteins which lack one or more of these domains. Such deleted proteins are useful in assays for identifying ligands and their binding activity.

5 In the rat wild type protein, amino acids 1-28 form a signal sequence; thus the mature proteins extend from amino acids 28-1162. The mature protein isoforms form yet another aspect of this invention. This differs somewhat from the signal sequence of 1-22 reported for mouse and human OB-R; the mature mouse and human isoforms form yet another aspect of this invention.

10 The OB-R isoform c', f or g gene can be introduced into virtually any host cell using known vectors. Preferred host cells include *E. coli* as well as mammalian and yeast cell lines.

One of ordinary skill in the art is able to choose a known vector which is appropriate for a given host cell; generally  
15 plasmids or viral vectors are preferred. The OB-R isoform c', f or g gene may be present in the vector in its native form, or it may be under the control of a heterologous promoter, and if desired, one or more enhancers, or other sequences known to regulate transcription or translation. The host cell containing the OB-R isoform c', f or g  
20 gene is cultured, and the OB-R isoform c', f or g gene is expressed. After a suitable period of time the OB-R c', f or g isoform protein may be harvested from the cell using conventional separation techniques.

A further aspect of this invention is the use of an OB-R  
25 c', f or g isoform in assays to identify OB-R c', f or g isoform ligands. A ligand binds to the OB-R isoform receptor, and *in vivo* may or may not result in an activation of the receptor. Ligands may be agonists of the receptor (i.e. stimulate its activity), antagonists (inhibit its activity) or they may bind with little or no effect upon the  
30 receptor activity.

In an assay for ligands, an OB-R isoform of this invention is exposed to a putative ligand, and the amount of binding is measured. The amount of binding may be measured in many ways; for example, a ligand or the OB-R isoform being investigated

- 7 -

may be labeled with a conventional label (such as a radioactive or fluorescent label) and then put in contact with the OB-R isoform under binding conditions. After a suitable time, the unbound ligand is separated from the OB-R isoform and the amount of ligand which has bound can be measured. This can be performed with any of the OB-R isoforms of this invention; alternatively the amount of binding of the various isoforms can be compared. In a competitive assay, both the putative ligand and a known ligand are present, and the amount of binding of the putative ligand is compared to the amount of binding to a known ligand. Alternatively, the putative ligand's ability to displace previously bound known ligand (or vice-versa) may be measured. In yet other embodiments, the assay may be a heterogeneous one, where the OB-R isoform may be bound to a surface, and contacted with putative ligands. Detection of binding may be by a variety of methods, including labelling, reaction with antibodies, and chomophores.

In another assay, the OB-R isoforms of this invention may be used in a "trans" activation assay. Such assays are described in U.S. Application Serial No. \_\_\_\_\_, Attorney Docket No. 19686PV, which was filed on April 22, 1996 and which is hereby incorporated by reference. In this assay, a cell which expresses an OB-R isoform of this invention (either naturally or through recombinant means) is transfected with a reporter gene construct comprising a minimal promoter, a leptin activation element and a reporter gene. Transcription of the reporter gene is dependant upon activation of the leptin activation element. Binding of a ligand to the receptor isoform activates the leptin activation element, which then allows transcription of the reporter gene.

The following non-limiting Examples are presented to better illustrate the invention.

- 8 -

EXAMPLE 1Preparation of mRNA and cDNA from rat tissues

Tissues were collected from lean and *falfa* Zucker rats and snap frozen in liquid nitrogen. The tissues collected included: hypothalamus, pituitary, lung, liver, kidney, heart, adrenal glands, smooth muscle, skeletal muscle, and adipose tissue. The tissues were homogenized with a Brinkmann Polytron homogenizer in the presence of guanadinium isothiocyanate. mRNA was prepared from hypothalamus, lung, and kidney according to the instructions provided with the messenger RNA isolation kit (Stratagene, La Jolla, CA). cDNA was prepared from approximately 2 µg of mRNA with the SuperScript™ choice system (Gibco/BRL Gaithersburg, MD). The first strand cDNA synthesis was primed using 1 µg of oligo(dT)12-18 primer and 25 ng of random hexamers per reaction. Second strand cDNA sythesis was performed according to the manufacturer's instructions. The quality of the cDNA was assessed by labeling an aliquot (1/10<sup>th</sup>) of the second strand reaction with approximately 1 µCi of [ $\alpha$ -<sup>32</sup>P]dCTP (3000 Ci/mmol). The labeled products were separated on an agarose gel and detected by autoradiography.

EXAMPLE 2Preparation of a hypothalamic cDNA library

Approximately 3.6 µg of phosphorylated *Bst* XI adapters (Invitrogen, San Diego, CA) were ligated to approximately 3 µg of cDNA prepared as described in Example 1. The ligation mix was then diluted and size-fractionated on a cDNA sizing column (Gibco/BRL Gaithersburg, MD). Drops from the column were collected and the eluted volume from the column was determined. An aliquot from each fraction was analyzed on an agarose gel. Fractions containing cDNA of greater than or equal to 1 kb were pooled and precipitated. The size-fractionated cDNA with the *Bst* XI adapters was ligated into the prokaryotic vector pcDNA II

- 9 -

(Invitrogen, San Diego, CA). The vector (4  $\mu$ g) was prepared for ligation by first cutting with the restriction endonuclease *Bst* XI, gel purifying the linearized vector, and then dephosphorylating the ends with calf intestinal phosphatase (Gibco/BRL, Gaithersburg, MD) according to the manufacturers instructions. The ligation contained approximately 10-20 ng of cDNA and approximately 100 ng of vector and was incubated overnight at 14°C. The ligation was transformed into 1 ml of XL-2 Blue Ultracompetent cells (Stratagene, La Jolla, CA) according to the manufacture's instructions. The transformed cells were spread on 133 mm Colony/Plaqué Screen filters (Dupont/NEN, Boston, MA), plated at a density of 30,000 to 60,000 colonies per plate on Luria Broth agar plates containing 100  $\mu$ g/ml Ampicillin (Sigma, St. Louis, MO).

15

### EXAMPLE 3

#### Screening a hypothalamic cDNA library

Colonies on filters were replica plated onto a second filter set. The master filter was stored at 4°C for subsequent isolation of regions containing colonies that gave a positive hybridization signal. The replica filters were grown for several hours at 37°C until colonies were visible and then processed for in situ hybridization of colonies according to established procedures (Maniatis, et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, Cold Spring Harbor, NY, which is hereby incorporated by reference). A Stratalinker (Stratagene, La Jolla, CA) was used to crosslink the DNA to the filter. The filters were washed at 55°C for 2 hours in 2x SSC and 0.5% SDS to remove bacterial debris. Eight to ten filters were then placed in a heat sealable bag (Kapak, Minneapolis, MN) containing 15-20 ml of 1x hybridization solution (Gibco/BRL, Gaithersburg, MD) containing 50% formamide and incubated for 1 hour at 42°C. The filters were hybridized overnight with greater than 1,000,000 cpm/ml of the radiolabeled probe described below in 1x

- 10 -

hybridization buffer (Gibco/BRL, Gaithersburg, MD) containing 50% formamide at 42°C. The probe, a 2.2 kb fragment encoding the extracellular portion of the Ob-R was labeled by random priming with [ $\alpha$   $^{32}$ P]dCTP (3000 Ci/mmol, Amersham, Arlington Heights, IL) using redi-prime (Amersham, Arlington Heights, IL). The probe was purified from unincorporated nucleotides using a Probequant G-50 spin column (Pharmacia Biotech, Piscataway, NJ). Filters were washed two times with 0.1x SSC 0.1% SDS at 60°C for 30 min and then subjected to autoradiography. Individual regions containing hybridization positive colonies were lined up with the autoradiogram of the hybridized filter. These were excised from the master filter, and placed into 0.5 ml Luria broth plus 20% glycerol. Each positive was replated at a density of approximate 50-200 colonies per 100 by 15 mm plate and screened by hybridization as previously described. Individual positive colonies were picked and plasmid DNA was prepared from an overnight culture using a Wizard kit (Promega, Madison, WI).

#### EXAMPLE 4

##### Amplification of Lean Rat OB-receptor cDNA using PCR

To provide for a probe to screen the hypothalamic cDNA library, the rat OB receptor was initially obtained by PCR using degenerate primers based on the mouse and human OB-receptor amino acid sequences. A set of oligonucleotide primers, were designed to regions with low codon degeneracy. The pairing of the forward primers ROBR 2 (5'-CAY TGG GAR TTY CTI TAY GT-3') and ROBR 3 (5'-GAR TGY TGG ATG AAY GG-3') corresponding to mouse amino acid sequences HWEFLYV and ECWMKG, with reverse primers ROBR 6 (5'-ATC CAC ATI GTR TAI CC-3'), ROBR 7 (5'-CTC CAR TTR CTC CAR TAI CC-3'), ROBR 8 (5'-ACY TTR CTC ATI GGC CA-3') and ROBR 9 (5'-CCA YTT CAT ICC RTC RTC-3') representing mouse amino acids, GYTMWI, VYWSNWS, WPMASKV, and DDGMKW provided good yields of the appropriately sized products. The fragments of interest

- 11 -

were amplified as long polymerase chain reaction (PCR) products by a modifying the method of Barnes (1994, *Proc. Natl. Acad. Sci.* 91:2216-2220, which is hereby incorporated by reference). In order to obtain the required long PCR fragments, Taq Extender (Stratagene, La Jolla CA) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) were used in combination. The standard PCR reaction mix, in a final volume of 20  $\mu$ l, contained 5 ng of template (lean rat cDNA), 100 ng of primers, 500  $\mu$ M dNTPs, 1 X Buffer 3 from the Expand kit, 0.1  $\mu$ l each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled reaction tubes. The amplification protocol was: 1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 3 min. using a Perkin-Elmer (Norwalk, CT) 9600 Thermal Cycler.

This strategy produced a series of PCR products with the largest being approximately 2.2 Kbp amplified from primers ROBR 2 and ROBR 9. These products were subcloned for DNA sequence analysis as described below. The insert was excised from the cloning vector with the restriction endonuclease *Eco* RI, and fragments were separated from the vector by agarose gel electrophoresis. The fragments were eluted from the gel using a Prep-A-Gene kit (BioRad, Richmond CA) according to the manufacturer's instructions and radiolabeled as described above.

#### EXAMPLE 5

##### Subcloning of PCR products

PCR products of the appropriate size were prepared for subcloning by separation on an agarose gel, excising the band, and extracting the DNA using Prep-A-Gene (BioRad, Richmond, CA). PCR products were ligated into pCR<sup>TM</sup>II (Invitrogen, San Diego, CA) according to the instructions provided by the manufacturer. The ligation was transformed into INVaF<sup>+</sup> cells and plated on Luria-Bertani plates containing 100  $\mu$ g/ml ampicillin and X-Gal (32  $\mu$ l of

- 12 -

50 mg/ml X-Gal (Promega, Madison, WI). White colonies were picked and grown overnight in Luria-Bertani broth plus 100 µg/ml ampicillin. Plasmid DNAs were prepared using the Wizard miniprep kit (Promega, Madison, WI). Inserts were analyzed by digesting the plasmid DNA with EcoRI and separating the restriction endonuclease digestion products on an agarose gel.

Plasmid DNA was prepared for DNA sequencing by ethanol precipitation of Wizard miniprep plasmid DNA and resuspending in water to achieve a final DNA concentration of 100 µg/ml. DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS. The initial DNA sequence analysis was performed with M13 forward and reverse primers, subsequently primers based on the rat OB-R sequence were utilized. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). DNA sequence data was analyzed with the Sequencher program.

- 13 -

WHAT IS CLAIMED IS:

1. *Ob*-receptor (OB-R) isoform c', f or g,  
substantially free from associated proteins.  
5
2. An OB-R isoform according to Claim 1 which is  
substantially pure.
3. An OB-R isoform according to Claim 1 which is a  
10 c' isoform.
4. An OB-R isoform according to Claim 1 which is  
an f isoform.
5. An OB-R isoform according to Claim 1 which is a  
15 g isoform.
6. An OB-R isoform according to Claim 1 which is  
from a rat.  
20
7. An OB-R isoform according to Claim 6 which is  
from a wild-type rat.
8. An OB-R isoform according to Claim 6 which is  
25 from a *fatty* rat.
9. An OB-R isoform according to Claim 3 which is  
human.
10. An OB-R isoform according to Claim 4 which is  
30 human.
11. An OB-R isoform according to Claim 5 which is  
human.

- 14 -

12. An OB-R isoform according to Claim 3 which is from a mouse.
13. An OB-R isoform according to Claim 4 which is from a mouse.
14. An OB-R isoform according to Claim 5 which is from a mouse.
15. A nucleic acid encoding an OB-R of Claim 1.
16. A nucleic acid according to Claim 15 which is a cDNA.
17. A vector comprising a nucleic acid which encodes an OB-R of Claim 1.
18. A vector according to Claim 17 which is a plasmid.
19. A host cell containing a vector according to Claim 17.
20. A host cell according to Claim 19 which is *E. coli*, a mammalian cell, or a yeast cell.
21. An assay to determine if a putative ligand binds to an OB-R isoform c', f or g comprising: contacting the putative ligand with an OB-R isoform c', f or g, and determining if binding has occurred.

- 15 -

22. An assay according to Claim 17 wherein the  
ligand is labeled.

23. An assay to determine if a putative ligand binds to  
5 an OB-R isoform c', f or g which is a trans-activation assay.

1/15

1 MTCQKFYVVL LHWEFLYVIT ALNLAYPTSP WRFKLFCA PP STTDDSF LSP  
51 AGVPNNTSSL KGASEALVEA KFNSTGIYVS ELSKTIFHCC FGNEQGGNCS  
101 ALTGNTGKT LASVVKPLVF RQLGVNWDIE CWMKGD LTLF ICHMEPL LKN  
151 PFKNYDSKVH LLYDLPEVID DLPLPLKDS FQTVQCNC SV RECECHVPVP  
201 RAKVNYALLM YLEITSAGVS FQSP LMSLQP MLVVKPDPPL GLRMEVTDDG  
251 NLKISWDSQT KAPFPLQYQV KYLENSTIVR EAAEIVSDTS LLVDSVLP GS  
301 SYEVQVRSKR LDGSGVWSDW SLPQLFTTQD VMYFPPKILT SVGSNASFCC  
351 IYKNENQTIS SKQIVMMNL AEKIPETQYN TVSDHISKVT FSNLKATRPR  
401 GKFTYDAVYC CNEQACHRY AELYVIDVNI NISCETDGYL TKMTCRWSPS  
451 TIQSLVGSTV QLRYHRRSLY CPDNPSIRPT SELKNCVLQT DGFYECVFPQ  
501 IFLLSGYTMW IRINHSLGSL DSPPTCVLPD SVVKPLPPSN VKAEITINTG  
551 LLKVSWEKPV FPENNLQFQI RYGLNGKEIQ WKTHEVFD AK SKSASLPVSD

FIG.1A

2/15

601 LCAVYVQVR CRRLDGLGYW SNWSSPAYTL VMDVKVPMRG PEFWRIMDGD  
651 ITKKERNVTLL LWKPLMKNDLS LCSVRRYVVK HRTAHNGTWS QDVGNQTNLT  
701 FLWAESAHTV TVLAINSIGA SLVNFNLTFSPMSKVNAVQ SLSAYPLSSS  
751 CVILSWTLSP NDYSLLYLVI EWKNLNDDDG MKWLRIPSNV NKYYIHDNFI  
801 PIEKYQFSLY PVFMEGVGKP KIINGFTKDD IAKQNDAGL YVIVPIIISS  
851 CVLLLGTLII SHQRMKKLFW DDVNPKNCS WAQGLNFQKP ETFEHLFTKH  
901 AESVIFGPILL LEPEPVSEEI SVDTAWKNKD EMVPAMVSL LLTTPDSTRG  
951 SICISDQCNS ANFSGAQSTQ GTCEDQCQSQ PSVKYATLVS NVKTVETDEE  
1001 QGAIHSSVSQ CIARKHSPLR QSFSSNSWEI EAQAFLLSD HPPNVISPQL  
1051 SFSGLDELLE LEGNFPEENH GEKSVYYLGV SSGNKKRENDM LLTDEAGVLC  
1101 PFFAHCLFSD IRILQESCSH FVENNLNLGT SGKNFVPYMP QFQSCSTHSH  
1151 KIENKMCIDL TV

FIG. 1B

3/15

1 TGGGGCAATT GGGCTGACCT TTCATTATGCT GGGATGTGCC TTGGAGGACT  
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT  
101 TTGTGTTAÇAC TGGGAATTTC TGTATGTGAT AACTGCACCT AACCTGGCCT  
151 ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA  
201 ACTGATGACT CCTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC  
251 TTTGAAGGGG GCTTCTGAAG CACTTGTGA AGCTAAATTT AATCAACTG  
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG  
351 AATGAGCAAG GTCAAAACTG CTCGGCACTC ACAGGCAACA CTGAAGGGAA  
401 GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA  
451 ACTGGGACAT AGAGTGCTGG ATGAAAGGG ACTTGACATT ATTCATCTGT  
501 CATATGGAAC CATTACTTAA GAACCCCTTC AGAATTATG ACTCTAAGGT  
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTG CCTCTGCCCC  
601 CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA  
651 TCGGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT

FIG. 2A

4/15

701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA  
751 TGTCACTGCA GCCCATGCTT GTTGGAAGC CCGATCCACC GCTGGGTTTG  
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA  
851 AACAAAAGCA CCATTTCAC TTCAATATCA GGTGAAATAT TTAGAGAATT  
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG  
951 GTAGACAGCG TGCTTCCCTGG GTCTTCATAC GAGTCCAGG TGAGGAGCAA  
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT  
1051 TTACCACACA AGATGTCAAG TATTTCCAC CCAAATTTCT GACGAGTGTT  
1101 GGATCCAATG CTTCCCTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT  
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG  
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTCTCTC  
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA  
1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA

FIG. 2B

5/15

1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA  
1401 ATGACTTGCA GATGGTCACC CAGCACAAATC CAATCACTAG TGGGAAGCAC  
1451 TGTGCAGTTG AGGTATCACA GGGCAGCCT GTACTGTCCC GATAATCCAT  
1501 CTATTCTGTC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC  
1551 TTTTATGAAT GTGTTTCCCA GCCAATCTTT CTATTATCTG GCTATACAAT  
1601 GTGGATCAGG ATCAACCAATT CTTTAGGTTG ACTTGACTCT CCACCAACGT  
1651 GTGTCCTTCC TGA CTCCGTA GTAAAACCA<sub>2</sub> TACCTCCATC TAATGTAAAA  
1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAAGCC  
1751 AGTCTTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG  
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA  
1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT  
1901 TCGCTGCCGG CCGTTGGATG GACTAGGTA TTGGAGTAAT TGGAGCAGTC  
1951 CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA  
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC

FIG. 2C

6/15

2051 CTTGCTTTGG AAGCCACTGA TGA AAAATGA CTC ACTGTGT AGTGTGAGGA  
2101 GGTATGTGGT GAAGCATCGT ACTGCCCCACA ATGGGACATG GTCACAAGAT  
2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC  
2201 TGTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA  
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC  
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC  
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA  
2401 ATGATGATGA TGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG  
2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT  
2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT  
2551 TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC  
2601 ATTGTACCGA TAATTATTTC CTCTTGTCCTG CTGCTGCTCG GAACACTGTT  
2651 AATTTCACAC CAGAGAAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC

FIG. 2D

7/15

2701 CCAAGAATTG TTCTTGGGCA CAAGGACTTA ATTTCCAAA GCCTGAAACA  
2751 TTGAGCATC TTTTACCAC GCATGCAGAA TCAGTGATAT TTGTCCTCT  
2801 TCTTCTGGAG CCTGAACCAG TTTCAGAAGA ATCAGTGTC GATACAGCTT  
2851 GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG  
2901 ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTA  
2951 CAGTGCTAAC TTCTCTGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG  
3001 AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG  
3051 AAAACAGTGG AACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG  
3101 CCAGTGCCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA  
3151 ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA

FIG. 2E

8/15

3201 CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT  
3251 GGAAC TGGAG GGAATTTTC CTGAAGAAA TCACGGGGA AAATCTGTGT  
3301 ATTATCTAGG AGTCTCCTCA GGAACA AAA GAGAGAA TATGCTTTTG  
3351 ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTCAG  
3401 TGACATCAGA ATCCTCCAGG AGAGTGTTC AACTTTGTA GAAATAATT  
3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT  
3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA  
3551 CTTAACTGTG TAATCTTGTG CAAAAC TTC CAGGTTCCAT TCCAGTAGAG  
3601 TGTGTCATGT ATAATATGTT CTTTATAGT TGTTGGTGGG AGAGAAAGCC

FIG. 2F

9/15

1 TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT  
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT  
101 TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACCT AACCTGGCCT  
151 ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA  
201 ACTGATGACT CCTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC  
251 TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG  
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG  
351 AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGAA  
401 GACGCTGGCT TCAGTGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA  
451 ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCACTGT  
501 CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGT  
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTG CCTCTGCCCC  
601 CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA

FIG. 3A

10/15

651 TCGGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT  
701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA  
751 TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG  
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA  
851 AACAAAAGCA CCATTTCCAC TTCAATATCA GGTGAAATAT TTAGAGAAAT  
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG  
951 GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGTCCAGG TGAGGAGCAA  
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTA CCTCAACTCT  
1051 TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT  
1101 GGATCCAATG CTTCCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT  
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG  
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTCTCC  
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA

FIG. 3B

11/15

1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA  
1351 TGGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACATAA  
1401 ATGACTTGCA GATGGTCACC CAGACAATC CAATCACTAG TGGGAAGCAC  
1451 TGTGCAGTTG AGGTATCACA GCGCAGCCT GTACTGTCCC GATAATCCAT  
1501 CTATTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC  
1551 TTTTATGAAT GTGTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT  
1601 GTGGATCAGG ATCAACCATT CTTAGGTTC ACTTGACTCT CCACCAACGT  
1651 GTGTCCCTCC TGA CTCCGTA GTAAACCAC TACCTCCATC TAATGTAAAA  
1701 GCAGAGATTA CTATAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC  
1751 AGTCTTTCCA GAGAAATAACC TTCAGTTCCA GATTCGATAT GGCTAAATG  
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA  
1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT

FIG. 3C

12/15

1901 TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC  
1951 CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCCTATGAG AGGGCCTGAA  
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC  
2051 CTTGCTTTGG AAGCCACTGA TGAATAATGA CTCACTGTGT AGTGTGAGGA  
2101 GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT  
2151 GTGGGAAATC AGACCAATCT CACTTTCCCTG TGGCAGAAT CAGCACACAC  
2201 TGTTACAGTT CTGGCCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA  
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC  
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC  
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA  
2401 ATGATGATGA TGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG  
2451 TATTATATCC ATGATAAATT TATTCCTATC GAGAAATATC AGTTTAGTCT

FIG. 3D

13/15

2501 TTACCCAGTA TTTATGGAAG GAGTTGAAA ACCAAAGATA ATTAATGGTT  
2551 TCACCCAAAGA TGATATCGCC AAACAGCAA ATGATGCAGG GCTGTATGTC  
2601 ATTGTAACCGA TAATTATTTC CTCCTGTGTC CTGCTGCTCG GAACACTGTT  
2651 AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC  
2701 CCAAGAAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GATAATGCCTG  
2751 GCAGAAATTA GAGGATATAG AGTGATGCC GTCAAATGCC TTTAGACTCT  
2801 GGCTTCCCTG GCTGTCTCAC ATCTCCCCTA TTGGAGCTAA GTGTGGTGCT  
2851 GTATTTAGCA GGGTATCTGG CAGATATTTT AAATTAATG AAATATCACC  
2901 CTAAATTTC AGATTCTGGT AAACCTGAAGT GAATTCAGA AATTATTGTA  
2951 TTTATGTGTG TGCACATATG TGTGCAGGTA CCCACCGAAA TCTGCAGAGG  
3001 CATCAGATGC CCCAGAGCTG GAACTGACAG TTGTGAGCCT GATATGAGTT  
3051 CTGGGAATGA GCTCAGTCCT CTGGAAGAGC TGCAAGCACT ATTAAGCTCT

FIG. 3E

14/15

3101 GAGCCATCTT TTCAGTCCCT CATGTATAGA TTAAAAAAA TTGGGGTTTG  
3151 AAGATCCTCA TTTGTGAGAA ATTCCTTCTT ACCTTTGCAA AACTTTTTC  
3201 TCATTTT TAG TATATGTATT CATATTTTAC TGTCTCATTT TCAATATATG  
3251 TGGTCACAGT TTTTAAGTAT TTCTAAGGCA TAACAAAGAT GTAATATTAA  
3301 GAATAAATAA AAGAATAAAT CAATAATCCA GATGGTAGTG ACAGACACCT  
3351 TTAATCCCAG TACTAAGGAG ACAGAGATAG GTAAATCTGT ATGAATTGA  
3401 GACACGCCCTG TTCTACAAAG AAATTTCAGG ACATCTAGGG GTATCCACAA  
3451 AGAAACACTG TCTCAAAAAA TGCCAAACAA TCAAAAAAAA AAAA

FIG. 3F

15/15

1 GTCAC TTTT AAGTATTAC CCAAGATATC TAAGGTGCA GTTAGATAC  
51 TCTATTACAT AGAGATCTTT AAACATCTTT AAAAGGCTTT ATTTGTCCCT  
101 GTTCACTTTA TTAATCCCGT TTATCCTTTG TCTATAGCAA TAGCTGGGTT  
151 TTGGATTTGA TCAGAGGAAA CAAAGTTCAG TCATTATCA CATGAGAGTT  
201 GACAAGGTGT CTTTTTTTTT TCTCGTCACT GTACATAAAA AAATAAATAC  
251 TACAAGAGGA AGGAACATTG TAGATGGAGA ATAGATAACT GACTAAAAGG  
301 GCTTTCCTTA GTCAAAAGT TTAGGATCAA TATTATGAGT TTCTGATATT  
351 CAATATTCA CCATGACTTA CAAGTACAGT GTTGTTTTT

FIG. 4

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/07521

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2.8, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
APS, BIOSIS, CA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	TARTAGLIA et al. Identification and expression cloning of a Leptin Receptor, OB-R. Cell. 29 December 1995, Vol. 83, pages 1263-1271, see entire document.	1-5, 9-20 ----- 1-23
X --- Y	CHUA et al. Phenotypes of Mouse <i>diabetes</i> and Rat <i>fatty</i> due to mutations in the OB (Leptin) Receptor. Science. 16 February 1996, Vol. 271, see pages 994-996.	1-8, 12-20 ----- 1-23
X --- Y	CHEN et al. Evidence that the diabetes gene encodes the Leptin Receptor: Identification of a mutation in the Leptin Receptor gene in <i>db/db</i> mice. Cell. 09 February 1996, Vol. 84, pages 491-495, see entire document.	1-5, 12-20 ----- 1-23

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

	Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A	document defining the general state of the art which is not considered to be of particular relevance		
E	earlier document published on or after the international filing date	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means		
P	document published prior to the international filing date but later than the priority date claimed	A	document member of the same patent family

Date of the actual completion of the international search  
19 JUNE 1997Date of mailing of the international search report  
71 JUL 1997Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

GARNETTE D. DRAPER

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/07521

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	CIOFFI et al. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nature Medicine. May 1996, Vol. 2, No. 5, pages 585-589, see entire document.	1-5, 9-20 ----- 1-23
X	WO 96/08510 A1 (PROGENITOR, INC.) 21 March 1996 (21.03.96), see the figures and claims.	1-23
X — Y	LEE et al. Abnormal splicing of the leptin receptor in <i>diabetic</i> mice. Nature. 15 February 1996, Vol. 379, pages 632-635, see entire document.	1-5, 12-20 ----- 1-23
X	HODGSON J. Receptor screening and the search for new pharmaceuticals. Bio/Technology. September 1992, Vol. 10, pages 973-997, see entire document.	21-23
X	CA 2,104,996 A1 (BEHRINGWERKE AKTIENGESELLSCHAFT) 01 March 1994 (01.03.94), see the claims.	21-23

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US97/07521

**A. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (6):

C12P 21/00; C12N 1/20, 15/00; G01N 33/53; C07H 21/04; C07K 1/00, 14/52; A61K 45/05, 38/19, 38/00

**A. CLASSIFICATION OF SUBJECT MATTER:**  
US CL :

435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2.8, 12